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HISTOLOGICAL CHANGES IN HYDRA VIRIDIS DURING REGENERATION.

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ALTHOUGH a great deal of work has been done on the regeneration of hydra, no one has as yet attempted to make out the histological changes that take place. This point would seem to be one of special interest, since the old piece appears to change its form as a whole to produce a new animal. The principal question to which I wished to find an answer was whether, during the period of regeneration, the old cells go over without change into the tissue of the new animal, or whether new cells are formed, and if so, in what part or parts.

Green hydras were used almost exclusively, since they regenerate more readily and with fewer abnormalities than does the brown species. Hydras of various lengths were taken, the foot end and circle of tentacles were cut away, and the remaining middle parts of the body wall were each divided by cross cuts into four—in some cases two—small rings. These were allowed to regenerate, and at different periods, ranging from immediately after the cutting to some days after the formation of new tentacles and foot, were killed in a solution of corrosive acetic, hardened, sectioned lengthwise, and stained in Delafield hæmatoxylin. In some cases the slides were dipped for a moment into a weak solution of picric acid in absolute alcohol, in order to differentiate the endodermal tissue.

Observation of the living piece showed that the ends became rounded, closing in from fifteen to sixty minutes after having been cut; that the piece then remained without permanent change of form for thirty or forty hours, although at intervals it might change its shape by expanding and contracting. At about this time small knoblike outgrowths, the tentacles, began to appear at one end, the other end fixed itself to the nearest

support, and in a few days the proportions of a normal hydra were assumed.

Serious difficulties arose in the microscopic study of the prepared sections, which it may be well to mention. Although undoubted karyokinetic divisions were seen in both neuromuscular and interstitial cells of the ectoderm, and in the endoderm, it was found almost impossible to determine the exact amount of dividing tissue, for the following reasons.

Throughout the entire period of regeneration there is an active production of new nettle cells, which are formed from interstitial cells of the ectoderm. This process begins by a slight sickle- or moon-shaped thickening of protoplasm along a part of the cell wall. In certain sections this thickening appears as a small, darkly stained rod which may easily be mistaken for a small nucleus in process of division. Again, in sectioning, this curved, thickened rod may be cut at such an angle as to bring distinctly into view only its extremities, producing the appearance of two small groups of darkly stained material. These could be distinguished from two separating groups of chromosomes only by bringing into focus the connecting line of the rod, which would lie slightly above or below the ends. In many cases it was found almost impossible to determine whether such a cell was forming a cnidocyst or was dividing karyokinetically. These difficulties were increased by the small size of many of the interstitial cells, by the fact that the wall of such cells is not sharply defined, and by the fact that at the time of the formation of nettle cells it is very often difficult to make out the nuclei of the changing cells.

Moreover, many nuclei of both neuromuscular and interstitial cells were found to be in a state that distinctly suggested activity of some sort, — the chromatin mass large and loose, or even in many interstitial cells broken and scattered throughout the nucleus. This appearance was very different from that of nuclei of typical resting cells in the same section, the latter containing one or two well-defined nucleoli. Yet the large number of nuclei with the chromatin scattered as just described, in sections containing comparatively few karyokinetic spindles, would seem to indicate that all such cells were not necessarily

dividing. Where so many cells were about to divide or had just completed division, we should expect to find a corresponding number undergoing the actual process. This, however, was not the case.

A further point should also be kept in mind, *viz.*, the possibility that multiplication of the interstitial cells may be concerned with the development of new nematocyst cells rather than with the regeneration of new tissue.

In order to get more light on these points, the tissue of a normal hydra was examined for comparison with that of the regenerating piece. Another hydra was irritated with a blunt needle in order to induce it to discharge the nettle batteries. It was then left undisturbed for about twenty hours, at which time it was killed and the tissue prepared as has been described. It was hoped by these means to obtain further light on the difficulties of the question, and to be able to distinguish more clearly between the phenomena of regeneration and those merely incidental to the formation of new nettle cells.

In the case of the stimulated hydra, nettle cells were found to be forming as in the regenerating pieces. Moreover, in the tissue of this hydra and of the undisturbed normal one also I was surprised to find undoubted evidence of cell division, in addition to the very general loose and broken appearance of the chromatin in some of the interstitial cells, which has been noted as occurring in the regenerating piece. This latter condition, found in a hydra upon which no operation had been performed, made it still less possible to consider such nuclei as dividing. On the other hand, a piece that had been regenerating seven days, and that had attained to the proportions of a normal polyp, was found to present the same appearance under the microscope. It is possible, therefore, that the normal tissue examined was that of a growing hydra, and that these conditions are common to the regenerating and growing forms, while further examination of the tissue of fully grown hydras might show a somewhat different state.

While these latter experiments, therefore, threw little light on the amount of division in the regenerating piece, yet the similarity in appearance between this regenerating tissue, the

tissue of the regenerating piece after the normal form had been attained, and the tissue of normal or growing hydra is of importance in a consideration of regeneration in this form.

I shall now attempt to make a conservative statement of the amount of dividing tissue in regenerating hydra, based on undoubted cell division.

There is very little evidence of the formation of new tissue during the early stages; an exception was noted in one piece killed five hours after the cutting, in which a number of divisions were observed in the ectoderm. Some few divisions can be observed before twenty hours, but from forty hours on, — the time of tentacle formation, — the division is more active and the number of dividing cells, especially neuro-muscular cells, is considerable. Division continues after the tentacles have begun. Spindles were seen in endodermal cells, and in neuro-muscular and interstitial cells. These divisions often occur in groups, especially in the neuro-muscular cells. In one case as many as five dividing nuclei of neuro-muscular cells were observed at one spot in a section cut from the side of the piece and so affording a surface view of ectoderm tissue alone. Fewer divisions were seen in the endoderm than in the ectoderm, partly, perhaps, because of the smaller number of cells in the endoderm.

Unlike the majority of cases of regeneration, however, the new cells are not formed at the cut surface alone, and the tentacles do not seem to be regenerated solely from new tissue. Divisions were found to occur as well at the sides as at the ends of the regenerating pieces, and in almost, if not quite, as great number. The first appearance of the tentacle, which in the living animal seems to be brought about by an outpushing of the tissue of the original piece, appeared under the microscope to be due very often to contraction in this region, causing a very slight protuberance of ectoderm, which could be fully accounted for by an outpushing of old material without the formation of new tissue. Divisions were sometimes seen at this place, sometimes not. Very frequently, however, in later stages, when the tentacle had grown to some length, there was seen to be a group of neuro-muscular cells dividing

at the base of the tentacle, and interstitial cells were seen to be dividing quite rapidly in the tentacle itself. It would seem, on the whole, that new tissue is furnished to the tentacle by division of cells at the proximal end, or base, rather than by division of cells that have already entered into the new organ.

With the exception of the formation of nettle cells from interstitial cells, it is not found that cells of one kind give rise to cells of another, but that neuro-muscular cells of the new hydra result from neuro-muscular cells of the piece, and interstitial from interstitial.

Considering, therefore, that the first appearance of the tentacle may be unaccompanied by cell division in its immediate neighborhood; that cell division, though frequently found in the region of the growing tentacle, is not confined to this region but is found elsewhere throughout the length of the regenerating piece; that division is found to be present as late as seven days after the cutting, that is, after the piece has assumed the proportions of a normal hydra; and that some division is observed in the tissue of the normal, probably rapidly growing hydra, — we are justified, I think, in concluding that the new cells which appear during the regeneration of hydra are formed by division of the old cells throughout the entire piece, as in the normally growing animal, and that the tentacles are formed from old cells and from cells that have arisen by division of the already differentiated cells of the old part.

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